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ORGANIC MICROCHEMISTRY

C. O. WILLITS AND C. L. OGG
Eastern Regional Research Laboratory, Philadelphia 18, Pa.

THE scope of the present review is essentially the same as that of other years. Judged from the number of papers written during the past year, there is no apparent lessening of interest on the part of microchemists in their efforts to improve existing methods of analysis. Noticeable is the increased attention being given both here and abroad to the subject of standardization.

APPARATUS

The accuracy, precision, and utility of the Cornwall syringe, Chaney pipet, and modified tuberculin syringe for delivering from 0.2 to 7.0 ml. of liquid were compared by Dern *et al.* (*29*). They concluded that syringes held the advantage in speed and accuracy and also in adaptability, because other than integral volumes could be measured and viscosity, surface tension, and temperature variations had less effect on the constancy of volumes delivered from syringes than from pipets. British Standards (*16*) specifies the design and dimensions of washout pipets for volumes from 0.1 to 1.0 ml. with tolerance and detailed methods for using the pipets. Flaschka (*41*) has described a combination microburet and Jones reductor which greatly simplifies titrations with solutions that must be kept in an inert atmosphere.

Two new melting point apparatus have been described, one by Hilbck (*61*), the other by Hippenmeyer (*62*). The apparatus of Hilbck uses exchangeable thermometers which increase the accuracy of reading when used over a wide temperature range.

The distillation and sublimation apparatus described by Erdos (*35*) is designed for use under reduced pressure and with small amounts of material. Conolly and Oldham (*27*) also described a distillation apparatus for quantities of 1 ml. or less.

A turntable for carrying out six simultaneous saponifications with reflux condensation was described by Grützner and Hintermaier (*52*). The modified Conway cell designed by Leurquin and Delville (*87*) has three compartments, one for sample, one for reagent, and another for absorbent solution, all covered by a glass plate. The sample and reagent can be mixed without contaminating the absorbent solution. A light, simple bomb for sodium peroxide fusions of micro, semimicro, or macro amounts of starting material was described by Wurzschmitt (*162*). With this bomb ethylene glycol was used in place of potassium nitrate and sugar, as the fusion temperature was lower and the metal of the bomb less affected.

Electrical apparatus described during the past year include a small high temperature, high vacuum furnace by Alberman (*4*), a simple, precise temperature regulator for electric furnaces by Lévy and Schick (*91*), an apparatus for microconductometric titrations of volumes less than 1 ml. by Stock (*135*), and a photoelectric micronephelometer designed for use with 0.1 to 0.5 ml. of solution by Mason (*98*).

Other apparatus included a drying tube described by Bradlow and Vander Werf (*13*), containers and manometers for micromanometric methods by Möhle (*101*), and a device for making pressure precipitation of sulfides by Stock and Heath (*136*).

Balances. Two articles published by Hodsman, one a report on a symposium on microchemical balances (*67*) and the other a discussion of developments in microchemical balance design (*66*), give a comprehensive picture of balance design, materials, and manipulation, as well as the sensitivity, precision, and practical application of results. Brown (*19*) described an improved 5-mg. aluminum foil rider for Ainsworth microbalances which reduced the rider seating errors. Komárek (*80*) discussed the origin of microbalances and Müller (*103*) presented an unpublished paper by Eigenberger in which proposals were made for improving the microlift balance for determining liquid densities. Improvements in the radioactive electronic microbalance were discussed by Feuer (*37*) and several modifications of torsion ultramicrobalances were reported by Korenman *et al.* (*82*). The magnetically controlled quartz fiber microbalance described by Edwards and Baldwin (*34*) had load capacities of 1.2 mg. unbalanced and 100 to 150 mg. balanced with a sensitivity of 0.1 microgram for 6 minutes of arc.

ELEMENTAL ANALYSIS

Carbon and Hydrogen. The determination of carbon and hydrogen in organic compounds continues to be the popular subject of analytical papers; achieving accuracy of analysis with simpler apparatus and technique is usually the goal. Kainz (*75*) has described an apparatus in which nitrous oxides are eliminated in the gravimetric procedure through the use of metallic copper. The advantage claimed for the copper absorbent is that it not only removes the oxides of nitrogen but eliminates the conditioning runs, as it is nonhygroscopic. Belcher and Ingram (*7*) advocate the use of manganese dioxide for the removal of oxides of nitrogen, and using their rapid oxygen flow technique this absorbent can be used outside the combustion tube and at room temperatures. For the slow or conventional combustion method they have described a modified combustion tube packing. The rapid combustion procedure continues to gain in popularity and Ingram (*70*) has described advantages of the rapid combustion process for carbon and hydrogen and outlined methods that have been successfully used in routine analysis. Wagner (*146*) has described a somewhat modified combustion tube which uses readily exchangeable extensions, the one without lead dioxide filling for compounds that do not contain nitrogen and the other with an aged lead dioxide filling for use when nitrogenous organic compounds are burned. He advocates not wiping absorption tubes prior to weighing, a point in accord with methods being adopted in the United States. With increasing interest in organofluorine compounds, it is but natural that studies have been made of methods for the successful analysis of these compounds. Belcher and Goulden (*6*) have proposed a method in which the sample is burned in a silica combustion tube but in a fairly slow stream of oxygen. The combustion products are further oxidized by passing them over platinum contacts at 750° C. Fluorine together with the other halogens and sulfur is removed by passing over silver wool also at 750° C. Any silicon tetrafluoride that may be formed is removed by granular sodium fluoride kept at 270° C. Reports of preliminary work indicate that fluorocarbons may be analyzed if water vapor is added to the combustion tube. The use of an external absorbent for nitrogen oxides is advocated.

Wurzschmitt (*161*), to avoid possible errors caused by the use of lead dioxide to absorb nitrogen oxides, has suggested the simultaneous determination of nitrogen and hydrogen. The hydrogen, measured as water, is absorbed in a tube placed between the combustion tube and the azotometer.

A statistical study of the factors affecting the accuracy and precision of the carbon and hydrogen microdetermination was reported by Ogg *et al.* (*105*). Treatment of the absorption tubes was the most important factor and the results indicated that the tubes should not be wiped and that the oxygen in the tubes should not be replaced by air. A method for the determination of carbon and hydrogen, based upon a statistical analysis of data obtained from the collaborative study of results from various methods, has been recommended by the referees of the Association of Official Agricultural Chemists as official "first action." The full details of the method are included (*156*). The British, like the Americans, have committees working on the standardization of microchemical apparatus and they have published (*17*) specifications of the component parts of the combustion train, including drawings showing dimensions as well as the quality of the glass and of the rubber tubing.

Several modifications of wet combustion procedures for carbon have been described. One which is applicable for the analysis of 10 to 15 mg. of biological materials, and which yields carbon values with a standard deviation of 1 to 2%, has been described by Hockenhull (*65*).

Charest *et al.* (*24*) have modified the apparatus of the Baker method, omitted potassium iodate from the Van Slyke–Folch reagent, and increased the concentration of chromium trioxide. Using the new apparatus and the modified reagent they have eliminated the cause of high values. A rapid method for the determination of carbon 14 has been described by Harper *et al.* (*54*). This is based upon the method of Entenmann using the Van Slyke–Folch oxidizing reagent. An apparatus is described in which the $C^{14}O_2$ absorption chamber also serves as the filter for the $BaC^{14}O_3$ precipitate. The dried precipitate can be counted with a windowless tube.

Kjeldahl Nitrogen. In the previous review it was noted from work of the authors that there is a minimum critical temperature for the digestion. White and Long (*152*) have proposed conducting the digestion in sealed tubes, which permits very high digestion temperatures and thereby shortens the time for completing the reduction. Jacobs (*72*) has emphasized the necessity of using a minimum of 0.7 to 0.8 gram of potassium sulfate per ml. of sulfuric acid. These amounts are in keeping with the recommended micromethod of the Association of Official Agricultural Chemists.

The popularity of the use of selenium as a catalyst in the digestion continues. Levin *et al.* (*88*) have established 3.5 mg. of selenium to 1 ml. of sulfuric acid as optimum for serum analysis. Ribas and López Capont (*112*) have developed a method using selenium and perchloric acid in the digestion mixture. Marzadro (*97*) has applied the Kjeldahl method to the analysis of compounds containing both ring nitrogen and side-chain nitrogen. The side-chain nitrogen is determined by the Kjeldahl method, the total nitrogen by a modified Dumas method, and the ring nitrogen is obtained by difference.

Sources of errors which can be avoided in the micro-Kjeldahl determination when boric acid is used to trap the ammonia have been shown by Wingo *et al.* (*159*) to be those due to boiling of the distillate prior to titration and "steaming out" the condenser Stetten's (*133*) study of the acidic behavior of boric acid solutio showed the amount of base required to neutralize boric acid solution of different concentrations to the methyl red end point. Although not a micro study, this paper is pertinent to the micro-Kjeldahl determination of nitrogen when boric acid is used as the ammonia absorbent and indicates the need for adjusting the ammonia–boric acid solution to constant volume before titration. The use of a solution of $NiSO_4(NH_4)_2SO_4 . 6H_2O$ as the absorbent for ammonia has been advocated by Blom and Schwarz (*10*); as its reaction with ammonia to form amines is rapid and a comparison solution is not necessary, the nickel salt does not interfere with the use of an acid-base indicator.

Several new designs of the Kjeldahl apparatus have been described. Hayazu (*56*) uses dry air instead of steam to sweep the ammonia from the reaction vessel. Leurquin and Delville (*86*) have modified the Van Slyke and Cullen aeration apparatus for the micro-Kjeldahl procedure. Hayazu (*58, 59*) has described a method using the Kjeldahl apparatus for the determination of the nitrite and nitrate ions. A simple distillation apparatus made from a wash bottle, a small-necked flask, absorption flask, and connecting tubes has been described by Möhlau (*100*) for use in the Kjeldahl procedure. Shepard and Jacobs (*127*) have modified the Parnas-Wagner apparatus so that the steam can be diverted from the steam generator without interruption. Klingmüller (*79*) has described an apparatus in which small amounts of ammonia can be distilled in 1 to 1.5 minutes.

Recommended specifications for Kjeldahl flasks, microdigestion rack, manifold, distillation apparatus (both Parnas-Wagner and one-piece model), and an electric steam generator have been published by the Committee on Microchemical Apparatus, Division of Analytical Chemistry, American Chemical Society (*134*).

The referees on microchemical methods, Association of Official Agricultural Chemists, through a statistical analysis of the data obtained by collaborative study of the Kjeldahl method, have recommended a method for adoption as official "first action" by the association (*157*). The investigation included studies of effect of potassium sulfate concentration in the digestion mixture.

Thompson and Morrison (*142*) studied the nesslerization method for determining nitrogen in plant material and determined the effect of such variables as stability of Nessler's solution, temperature and alkalinity effects, and conditions of sample digestion.

Dumas Nitrogen. A modified apparatus for the determination of nitrogen by the Dumas procedure has been described by Dirscherl and Wagner (*32*); the combustion is made in an electric furnace moved with an automatic device. They have also described a modified Kipp generator which they claim will deliver microbubbles even after lengthy periods of idleness.

Specifications for the dimensions of a 1.5-ml. micronitrometer for use with the Dumas combustion train have been established by the British Standards Institute (*18*).

A new method for the determination of nitrogen in organic materials by hydrogenation has been described by Gel'man and Korshun (*45*). The samples are hydrogenated in a rapidly flowing stream of hydrogen in the presence of an iron or nickel catalyst at temperatures of 300° to 400° C. Best results were obtained with the iron catalyst. The evolved ammonia is determined iodometrically with potassium hydrogen iodate and potassium iodide in the absorption liquid and by back-titration with sodium thiosulfate. Through study of the reaction of a mixture of concentrated hydrochloric and nitric acids and monocarboxylated α-monoamines, Renard and Deschamps (*111*) have shown that the volume of gas liberated corresponds to the nitrogen of the organic material according to the equation:

$$R\text{—}CHNH_2\text{—}COOH + HCl + HNO_2 \longrightarrow R\text{—}CHCl\text{—}COOH + N_2O + 2H_2O$$

Provided the compound studied does not set up secondary reactions with nitric acid, the proposed method provides a means of determining amine nitrogen of α-amino acids and α-amino sulfonic acids and the amide nitrogen of amides and sulfonamides. Sternglanz *et al.* (*132*) reported a modification and simplification of Zimmermann's Dumas method for nitrogen. Tractable samples were mixed with fine copper oxide in a porcelain boat before being placed in the combustion tube, whereas copper oxide plus cobaltic oxide was used if the material was refractory. Mangeney (*94*) also has described modification of Zimmermann's automatic combustion method for nitrogen in organic compounds. The modified method is equally applicable to micro and semimicro sized samples.

Wurzschmitt (*160*) like other European microanalysts believes that better hydrogen analysis values can be obtained when the hydrogen is determined as a part of the Dumas nitrogen procedure rather than as part of the dry catalytic carbon procedure.

Phosphorus. A titrimetric method for the determination of phosphorus has been described by Sakamoto and Hayazu (*121*). The sample is wet ashed and the phosphorus separated as the molybdate, from which ammonia is evolved by the micro-Kjeldahl procedure. The titer of the standard acid is in terms of phosphorus. Diemair and Baier (*31*) proposed a similar titrimetric procedure which they applied to the determination of phosphorus in growing yeast. Tsuzuki *et al.* (*143*) established a new factor for phosphorus when determined by the micro-Lorenz method of weighing directly the precipitated ammonium phosphomolybdate.

An amperometric titration method for phosphorus has been described by Boos and Conn (*11*) in which the phosphate in the solution of the carbonate fusion products of the phosphorus compound is titrated with 0.1 M uranyl acetate at -0.6 volt.

Sterges *et al.* (*131*) have modified the A.O.A.C. official method for phosphorus in plants by adding sulfuric and perchloric acids to the digestion mixture and using the same time for color development in the test sample as for the standard. These changes have shortened the time of analysis and increased the accuracy of the method.

Oxygen. Unterzaucher (*144*) has written a review of experiences gained from 10 years' use of the blank-free iodometric microdetermination of oxygen. The method of Unterzaucher permits the determination of micro, semimicro, and trace quantities of oxygen. For constitutional oxygen 3- to 5-mg. samples are required. The presence of fluorine and phosphorus interferes. The author maintains that his method is characterized by complete freedom from a blank, a view not shared by many American microanalysts. Unterzaucher believes the cause of blanks to be use of impure reagents, inconsistency of temperature of carbon contact, and use of insufficiently cleaned reaction tubes or those whose inner surfaces are damaged. Using this same procedure he has proposed a new method for the determination of carbon and hydrogen. The water from the hydrogen is retained on anhydrous barium chloride, and after the carbon has been determined iodometrically, the oxygen in the water, liberated from the barium chloride, is also determined iodometrically. This method is claimed to give greater accuracy for the carbon and hydrogen than is obtainable by the usual dry-catalytic-oxygen-combustion method. Grant *et al.* (*51*) have made improvements in the iodine pentoxide method for the estimation of carbon monoxide at low concentrations through refinements in the apparatus and by measurement of the liberated iodine spectrophotometrically. Deinum and Schouten (*28*) have described a semimicromethod for the direct determination of oxygen in organic compounds. The details of the method are essentially the same as those described by Unterzaucher.

Sulfur. Walter (*149*) has developed a photometric method for detecting the end point in the titrimetric method for sulfur, using as indicator tetrahydroxyquinone or the alkali salt of rhodizonic acid. Agazzi *et al.* (*3*) and Siegfriedt *et al.* (*128*) both described a small scale oxygen bomb for the determination of sulfur. Agazzi followed the combustion by a gravimetric determination of the sulfate formed, whereas Siegfriedt used the volumetric method employing tetrahydroxyquinone as indicator. A method for the determination of sulfur applicable to all types of organic compounds regardless of the sulfur linkage has been described by Roth (*115*). The sulfur in the compound is oxidized to sulfate in a microbomb tube filled with oxygen. The sulfate is reduced in a formic acid–acetic acid mixture and the hydrogen sulfide is carried by a stream of hydrogen to zinc acetate. The zinc sulfide is converted to hydrogen sulfide, which is determined photometrically from the reduction of methylene blue. For those who are interested in the benzidine method for sulfur determination, Fischer and Sprague (*40*) have shown by means of electron micrographs that the incomplete precipitation of benzidine sulfate in the presence of foreign ions is due to the imperfect crystal formation and growth. A possible explanation of the observed phenomena is based on the Paneth-Fajans-Hahn law. Fischer with Chen (*39*) has also shown that small quantities of sulfate of the order of 0.005 N can be determined by direct conductometric titration with standard benzidine dihydrochloride solution. Phosphate ions interfere but can easily be removed.

Hayazu (*57*) and Bussmann (*22*) have described applications of the Zimmermann volumetric sulfur method as well as causes of error that may occur.

A new volumetric method for sulfur has been described by Padowetz (*107*), in which the sulfuric acid produced is precipitated by adding a measured excess of barium chloride and the excess barium chloride is measured by argentometric titration of the chloride ion after the hydrochloric acid formed has been driven off.

An apparatus for the rapid air oxidation of sulfur-containing organic compounds, in which the sample is burned in a tube with an oxidation catalyst packing, has been described by Wilson and Straw (*158*). The catalyst used is vanadium pentoxide on alumina.

A titrimetric method for sulfur, based upon the determination of the amount of barium in excess of that precipitated by the sulfate, in which barium diphenylaminosulfate is used as the indicator and standard Mohr's salt as the titrant to measure the chromate ions in excess of those that react with the excess barium ions, has been described by Tamiya (*138*). Zinneke (*164*) has developed a titrimetric sulfur method in which the organic sulfur compound is burned in a stream of oxygen and the combustion product is passed over hot platinum and then over silver gauze. The latter reacts with the sulfur, forming a coating of silver sulfate. This is dissolved in water and titrated with standard potassium iodide in the presence of nitrite and starch. Sakamoto, Hayazu, and Takenaka (*122*) have advocated the use of wet oxygen in the "dry" catalytic sulfur combustion method.

Rosenberg *et al.* (*114*) have modified the amperometric titration method for sulfhydryl groups. They used a vibrating electrode as a combination electrode and stirrer, and were able to measure amounts of sulfhydryl as small as 1 microgram.

Halogen. A method has been proposed by Gatterer (*43*) for the rapid and reliable determination of chlorine, bromine, iodine, and sulfur. The sample is subjected to a high frequency electromagnetic field and the spectrum is photographed and evaluated in the usual way. Detection is possible down to about 0.001% and quantitative estimation is possible down to 0.1 to 0.033% with an accuracy of 5 to 10%.

The use of sodium peroxide fusions continues to be popular in the determination of halogens. Lévy (*90*) outlines such a method using the Parr bomb for the mineralization of the halogens, which are subsequently determined by his method of potentiometric argentometry by use of equivalence potentials. Martin (*95*) has shown that there is still need for suitable rapid and selective methods for the microchemical determination of the halogens. While he recognizes the advantages of the Parr bomb micromethod for the destruction of organic substances, he calls attention to the fact that exceptionally close maintenance of the experimental conditions must be observed and that these conditions are yet to be perfected. Agazzi *et al.* (*3*) determined chlorine as well as sulfur in the small scale oxygen bomb described by them. A simplified gravimetric method by which chlorine, bromine, and iodine of organic compounds can be determined has been devised by Safford and Stragand (*118*). The halogen gases, products of dry oxygen-catalytic combustion, are absorbed by a silver gauze. The resulting gain in weight of the gauze by the formation of the silver halides represents directly the halogens of the sample. A potentiometric method for measuring the halogens in a solution of the fusion mixture formed in a Parr bomb has been described by Lévy (*89*). Pääbo and Rottenberg (*106*) have replaced the indirect alkalimetric method of Zacherl and Krainick with a direct mercurimetric titration using diphenylcarbazide as the indicator. The method has been applied to halogenated hydrocarbons and fatty acids. When the sample contains sulfur the results are not satisfactory. A titrimetric method for the simultaneous determination of the halogens and sulfur in catalytic oxidation combustion products has been outlined by Wagner and Bühler (*147*). An argentometric method for the determination of chlorine, bromine, and thiocyanate using a back-titration with potassium iodide and an adsorption indicator of either *p*-ethoxy or *p*-methoxychrysoidine has been suggested by Schulek and Pungor (*126*). Sakaguchi (*120*) has listed eight indicators for the argentometric titration of chlorine, bromine, and iodine and has given the relative sensitivities of each indicator.

The success and failure of the use of the silver–silver chloride electrode for the potentiometric determination of chlorine in biological and nonbiological fluids were discussed by Duxbury (*33*). Viswanathan (*145*) has outlined a titrimetric ultramicroprocedure for the determination of chlorine.

A method for the determination of bromine, in which the bromine in an organic compound is converted to a bromide by heating with metallic sodium and absolute alcohol and the bromide is measured photometrically by the amount of orange-yellow color developed upon the addition of gold chloride, has been described by Desassis and Macheboeuf (*30*). Brandt and Dahlenborg (*15*) have suggested the advantages to be gained by using ultraviolet light when titrating iodine in a two-layer system, acetic acid–water, and chloroform containing thioflavin as the fluorescence indicator. The chloroform solution will not show full fluorescent brilliance until all the iodine has been reduced to iodide.

A method giving excellent results for the determination of iodine in 3- to 4-mg. samples is that of Kainz (*76*), in which the sample is heated with dry potassium, yielding potassium iodide. This is dissolved in water and oxidized with bromine, potassium iodate and iodide are added, and the iodine liberated from the acidified solution is titrated with thiosulfate. Nesh and Peacock (*104*) have used carrier-free radioactive iodine in Baker's method for the determination of protein-bound blood iodine.

The current interest in fluorinated compounds has intensified the need for analytical methods for fluorine analysis. Three papers dealing with this subject have appeared recently. Clark (*26*) has outlined a simple procedure in which the fluorinated organic compound is burned in a stream of oxygen passing over a platinum catalyst in a quartz tube at 900° C. The fluorine is determined by an acid-base titration of the combustion products. Necessary precautions and limitations of the method are discussed. Rickard *et al.* (*113*) have described a method of determining fluorine in solid halocarbons. The sample is decomposed at 1100° C. in a stream of moist oxygen and the fluorine is determined colorimetrically with standard ferric salicyclic acid. The combustion tube can be of quartz, platinized quartz, or platinum. Williams (*154*) is able to detect as little as 1 microgram of fluorine. The halogen is converted to hydrogen fluoride which acts upon glass, silicon from the glass is dissolved, and hydrated silicon dioxide is deposited on the glass by hydrolysis of the silicon tetrafluoride. Optimum conditions are given and a useful apparatus is described.

Other Elements. Kahane and Korach (*74*) described a method for determining selenium in small amounts of seleniferous organic matter. After decomposition by acid digestion the selenium was reduced in a strongly acid medium by sulfur dioxide and weighed as the black selenium.

Mercury was determined by Hirai and Hayazu (*63*) by carrying out a Kjeldahl digestion of the organic matter and then titrating the divalent mercury with thiocyanate by the Volhard method.

Kingsley and Schaffert (*78*) described a method for determining arsenic in samples containing less than 0.1-microgram quantities and discussed the results of applying the method to biological materials.

A micromethod for the boric ion developed by Gautier and Pignard (*44*) depends on the formation of a barium borotartrate complex and can be applied to the determination of boron in inorganic, organic, or biological materials.

A modification of the Lewis-Marmoy method for potassium was reported by Chapman (*23*) for determining potassium in leaf ash. In this volumetric semimicromethod the potassium as the cobaltinitrite in aqueous 30% disodium acid phosphate is titrated with standard permanganate solution.

To ensure that all the ash in a sulfate ash determination exists as the sulfate, Martin (*96*) has suggested that ashing be done as usual in a platinum boat but that ignition be done in a stream of oxygen containing sulfur dioxide gas. The mixture forms sulfuric anhydride in contact with the platinum, which serves as catalyst.

GROUP ANALYSIS

Lithium aluminum hydride was used by Schöniger (*124*) to liberate active hydrogen from 2- to 5-mg. samples. The hydrogen formed was first oxidized to water and the oxygen of the water determined by the Unterzaucher procedure. Schöniger (*123*) described a method for determining unsaturation by catalytic hydrogenation. Ruziczka (*117*) discussed the existing methods for the microdetermination of iodine value and recommended the Gorbach microburet for such determinations. Semimicromethods for both iodine value and saponification number were described by Komori (*81*) using approximately 30 and 100 mg., respectively, of oil or fat. Determination of carbonyl groups with 2,4-dinitrophenylhydrazine was described by Schöniger and Lieb (*125*). Gorbach (*46*) gave directions for determining the carbonyl number of fats with hydrazine hydrochloride using 0.5- to 2.5-mg. samples.

MOLECULAR WEIGHT

A new, less fragile apparatus for determining molecular weights by the isothermal distillation method was described by Hoyer (*68*), who used the apparatus with as little as 0.4 mg. of sample. Hawkins and Arthur (*55*) designed an apparatus which works equally well for determining the molecular weight of both high and low boiling liquids by the vapor density method. According to Brandstätter (*14*), cryoscopic molecular weight determinations can be conducted in preparations placed between the slide and cover glass on the hot-stage microscope. Camphor is too volatile for this method but cyclopentadecanone (Exaltone), bornyl bromide, or camphene may be used.

MOISTURE

Microdetermination of moisture by Karl Fischer titration was the subject of two papers. Wiberley (*153*) described an improved procedure using a special 1-ml. buret and a technique for preserving the reagent so that only weekly standardizations were usually necessary. Ciusa and Moroni (*25*) were skeptical of the feasibility of using Karl Fischer reagent for moisture but state that the method can be recommended for precise microanalyses, if certain precautions are observed.

Sugar. Collaborative results reported by Goss (*49*) showed that Somogyi's micromethod for reducing sugars is satisfactory. Fonty (*42*) recommended the use of polyvinylpyrrolidone or the polyoxyalkylene derivative of sorbitan mono-oleate (Tween 80) to stabilize the suspensions for the Folin-Malmos micromethod for sugar. A review of micromethods for sugar in blood was published by Accoyer (*1*). Small quantities of a mixture of glucose, maltose, and dextrins were analyzed colorimetrically by Rüggeberg (*116*) with an average error of $\pm 4\%$. Micromethods for serum amylase and blood glucose were described by Teller (*141*) and Accoyer *et al.* (*2*), respectively.

Fatty Acids. Electrometric titration of organic acids was discussed by Ingold (*69*), who used the glass electrode as pH indicator and titration vessel. He recommended the use of tetramethyl ammonium hydroxide in place of alkali hydroxides and glycol monomethyl ether as solvent for non-water-soluble materials. Microtitrations of fatty acid in a mixture of chloroform, ether, and alcohol as solvent were reported by Gracián Tous and Vioque Pizarro (*50*) with errors of about 5% for 5-mg. samples. The microchemical determination of the amino acids in proteins using only 200 to 300 mg. of sample was described by Gorbach (*48*). The analysis requires about 13 hours and includes all recognized essential amino acids. The analysis of certain amino acids separated by paper chromatography was described by Pereira and Serra (*110*). Other methods for organic acids include one for lactic acid by Lang and Pfleger (*85*), two for citric acid by Taussky (*140*) and Buffa (*20*), and a method for picric acid in picrates of organic bases by Stohr and Scheibl (*137*). A review of the micromethods for *p*-aminosalicylic acid with detailed procedures was published by Stelt (*130*).

Miscellaneous. The colorimetric determination of ethyl alcohol in amounts as low as 1 microgram in 1 ml. of solution was reported by Williams and Reese (*155*). The amount of dichromate consumed in the oxidation of the alcohol was measured by determining the excess dichromate after complexing with *s*-diphenyl carbazide. The 8-quinolinol complex of vanadium was used by Buscarons *et al.* (*21*) to determine alcohols in either organic or aqueous solution.

Free formaldehyde was determined on a micro scale by a spectrophotometric procedure developed by Tanenbaum and Bricker (*139*) employing the time-sensitive reaction between the aldehyde, phenylhydrazine, and potassium ferricyanide. The determination of formaldehyde with chromotropic acid was used by Lambert and Neish (*84*) as a means of determining glycerol in fermentation solutions after the latter had been oxidized with periodic acid. Bradbury (*12*) determined glycerol by reacting it with hydriodic acid to liberate iodine, which finally is determined by thiosulfate titration. Andrade and Suárez (*5*) compared three different methods for determining glycerol and other polyalcohols separately and in mixtures with ethanol, glycol, and sugars. Three pairs of workers—Hirt and Gisclard (*64*), Gunther and Blinn (*53*), and Ketelaar and Hellingman (*77*)—reported methods for determining the insecticide parathion, now being used extensively. A micromethod for the determination of fat reported by Jones (*73*) makes use of the formation of monolayer films and measurement of their areas.

REVIEWS AND GENERAL PAPERS

The fifteen or more general papers and reviews show that this has been a year for summing up, both in the general field of microchemistry and in special fields of application. A review by Benedetti-Pichler (*8*) covered both organic and inorganic microchemistry in the United States, while that by Zacherl (*163*) discussed the situation of microchemistry in Austria. Hecht (*60*) attempted to deduce the trend in microchemistry from the nature of the lectures presented at the 1950 International Microchemical Congress.

Parks and Lykken (*109*) discussed reduced-scale (micro and semimicro) methods of analysis for most of the determinations commonly made in organic analysis. They also reviewed (*108*) the potentiometric, amperometric, and polarographic methods of microanalysis.

The role of microchemistry in the field of biochemistry was discussed by Ley (*92*) including physical instruments, chemical (Pregl) methods, and manometric (Warburg) methods. Microchemistry in practical pharmacy was the subject of an article in which Lüdy-Tenger (*93*) discussed both physical and chemical tests he has found useful. Gorbach (*47*) discussed the application of microchemistry to the fat field and Waldmann (*148*) considered the determination of vitamins as a microchemical problem.

The elemental analysis of nitrogen-containing organic compounds was the subject of a review by Moelants (*102*), in which he considered the various combustion methods. Ishizaka (*71*) reviewed microdiffusion analysis by the Conway method, while Sakaguchi (*119*) discussed the use of organic reagents in microanalysis.

Other general articles bearing on microchemistry were the role of microchemistry in the new fields of analytical chemistry by Wenger (*150*), microchemical applications of catalytic and induced reactions by West (*151*), and a general discussion of spot test analysis by Feigl and West (*36*).

A discussion of the points and problems involved in attempting to reduce the scale of an analytical reaction was presented by Benedetti-Pichler (*9*). Although not specifically for microchemistry, the tables showing the percentage compositions of organic compounds arranged by Kuffner and Gruber (*83*) should be an aid to microanalysts.

Sobotka (*129*) pointed out that the greatest need in microchemistry was a systematic development of micromethods for determining physical constants. The microdetermination of critical mixing temperatures observed on a Kofler hot stage was used by Fischer (*38*) to identify, test the purity of, and quantitatively determine organic liquids. He also listed the different physical micromethods used for liquid examination on the hot stage. Milazzo and Paoloni (*99*) found *o*-dianisidine an effective redox indicator for microtitrations.

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LITERATURE CITED

(1) Accoyer, P., *Ann. biol. clin.* (*Paris*), 8, 367–81 (1950).
(2) Accoyer, P., Camelin, A., Garnung, H., and Bernard, *J. méd. Lyon*, 28, 301–3 (1947).
(3) Agazzi, E. J., Parks, T. D., and Brooks, F. R., ANAL. CHEM., 23, 1011 (1951).
(4) Alberman, K. B., *J. Sci. Instruments*, 27, 280–2 (1950).
(5) Andrade, J. L., and Suárez, A. M., *Rev. farm.* (*Buenos Aires*), 92, 8–19 (1950).
(6) Belcher, R., and Goulden, R., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 679–89 (1951).
(7) Belcher, R., and Ingram, G., *Anal. Chim. Acta*, 4, 401–10 (1950).
(8) Benedetti-Pichler, A. A., *Angew. Chem.*, 63, 158–62 (1951).
(9) Benedetti-Pichler, A. A., *Mikrochemie ver Mikrochim. Acta*, 36/37, 38–53 (1951).
(10) Blom, Jakob, and Schwarz, Birgit, *Acta Chem. Scand.*, 3, 1439–40 (1949).
(11) Boos, R. N., and Conn, J. B., ANAL. CHEM., 23, 674–5 (1951).
(12) Bradbury, R. B., *Mikrochemie ver. Mikrochim. Acta*, 38, 114–19 (1951).
(13) Bradlow, H. L., and Vander Werf, C. A., *Chemist-Analyst*, 39, 71 (1950).
(14) Brandstätter, Maria, *Mikrochemie ver. Mikrochim. Acta*, 36/37, 291–5 (1951).
(15) Brandt, Knut, and Dahlenborg, Hans, *Acta Chem. Scand.*, 4, 582–8 (1950).
(16) *Brit. Standards*, 1428, Pt. D2 (1950).
(17) *Ibid.*, Pt. Al (1950).
(18) *Ibid.*, Pt. D3 (1950).
(19) Brown, L. E., ANAL. CHEM., 23, 388 (1951).

(20) Buffa, Aldo, *Ann. chim. (Rome)*, 40, 617–19 (1950).
(21) Buscarons, F., Marín, J. L., and Claver, J., *Anal. Chim. Acta*, 3, 417–21 (1949).
(22) Bussmann, G., *Helv. Chim. Acta*, 33, 1566–8 (1950).
(23) Chapman, G. W., *J. Agr. Sci.*, 37, 29–31 (1947).
(24) Charest, M. P., Koch, P., and Gagnon, A., *Rev. Can. Biol.*, 9, 422–5 (1951).
(25) Ciusa, W., and Moroni, E., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 273–5 (1951).
(26) Clark, H. S., ANAL. CHEM., 23, 659–61 (1951).
(27) Conolly, J. M., and Oldham, G., *Analyst*, 76, 52–3 (1951).
(28) Deinum, H. W., and Schouten, A., *Anal. Chim. Acta*, 4, 286–99 (1950).
(29) Dern, R. J., Pullman, T. N., and Williams, H. R., *J. Lab. Clin. Med.*, 36, 494–500 (1950).
(30) Desassis, A., and Macheboeuf, M., *Ann. inst. Pasteur*, 76, 6–15 (1949).
(31) Diemair, W., and Baier, R., *Z. anal. Chem.*, 133, 7–12 (1951).
(32) Dirscherl, A., and Wagner, H., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 628–33 (1951).
(33) Duxbury, McD., *Analyst*, 75, 679–84 (1950).
(34) Edwards, F. C., and Baldwin, R. R., ANAL. CHEM., 23, 357–61 (1951).
(35) Erdos, José, *Mikrochemie ver. Mikrochim. Acta*, 36/37, 417–19 (1951).
(36) Feigl, F., and West, P. W., *Ibid.*, 36/37, 191–205 (1951).
(37) Feuer, I., *Ibid.*, 35, 419–30 (1950).
(38) Fischer, R., *Ibid.*, 36/37, 296–301 (1951).
(39) Fischer, R. B., and Chen, W. K., *Anal. Chim. Acta*, 5, 102 (1951).
(40) Fischer, R. B., and Sprague, R. S., *Ibid.*, 5, 98–101 (1951).
(41) Flaschka, H., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 269–72 (1951).
(42) Fonty, P., *Ann. biol. clin. (Paris)*, 8, 312–15 (1950).
(43) Gatterer, A., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 476–85 (1951).
(44) Gautier, J. A., and Pignard, P., *Ibid.*, 36/37, 793–800 (1951).
(45) Gel'man, N. E., and Korshun, M. O., *Doklady Acad. Nauk S.S.S.R.*, 72, 895–8 (1950).
(46) Gorbach, G., *Fette u. Seifen*, 52, 405–6 (1950).
(47) *Ibid.*, 53, 3–9 (1951).
(48) Gorbach, G., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 902–11 (1951).
(49) Goss, B. K., *J. Assoc. Offic. Agr. Chemists*, 33, 300–1 (1950).
(50) Gracián Tous, J., and Vioque Pizarro, A., *Anales real soc. españ. fís. y quím.*, 46B, 105–10 (1950).
(51) Grant, G. A., Katz, M., and Haines, R. L., *Can. J. Technol.*, 29, 43–51 (1951).
(52) Grützner, R., and Hintermaier, A., *Mikrochemie ver. Mikrochim. Acta*, 38, 66–7 (1951).
(53) Gunther, F. A., and Blinn, R. C., ANAL. CHEM., 22, 1450 (1950).
(54) Harper, P. V., Jr., Neal, W. B., Jr., and Rogers, G. R., *J. Lab. Clin. Med.*, 36, 321–4 (1950).
(55) Hawkins, J. J., and Arthur, P., ANAL. CHEM., 23, 533–4 (1951).
(56) Hayazu, R., *J. Pharm. Soc. Japan*, 70, 357–8 (1950).
(57) *Ibid.*, pp. 741–2.
(58) *Ibid.*, 71, 135–6 (1951).
(59) *Ibid.*, pp. 207–8.
(60) Hecht, F., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 1181–8 (1951).
(61) Hilbck, H., *Ibid.*, 36/37, 307–11 (1951).
(62) Hippenmeyer, F., *Pharm. Acta Helv.*, 25, 161–8 (1950).
(63) Hirai, M., and Hayazu, R., *J. Pharm. Soc. Japan*, 70, 670–1 (1950).
(64) Hirt, R. C., and Gisclard, J. B., ANAL. CHEM., 23, 185–7 (1951).
(65) Hockenhull, D. J. D., *Biochem. J.*, 46, 605–6 (1950).
(66) Hodsman, G. F., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 133–42 (1951).
(67) Hodsman, G. F., *Roy. Inst. Chem. Lectures Monographs Repts.*, No. 4, 5–15 (1950).
(68) Hoyer, H., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 1169–73 (1951).
(69) Ingold, W., *Ibid.*, 36/37, 276–82 (1951).
(70) Ingram, G., *Ibid.*, 36/37, 690–705 (1951).
(71) Ishizaka, O., *Kagaku no Ryôiki (J. Japan. Chem.)*, 4, 591–8 (1950).
(72) Jacobs, M. B., *J. Am. Pharm. Assoc.*, 40, 151–3 (1951).
(73) Jones, K. K., *Quart. Bull. Northwestern Univ. Med. School*, 24, 253–6 (1950).
(74) Kahane, E., and Korach, S., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 781–9 (1951).
(75) Kainz, G., *Ibid.*, 35, 569–71 (1950).
(76) *Ibid.*, pp. 466–72.
(77) Ketelaar, J. A. A., and Hellingman, J. E., ANAL. CHEM., 23, 646–50 (1951).
(78) Kingsley, G. R., and Schaffert, R. R., *Ibid.*, 23, 914–19 (1951).
(79) Klingmüller, V., *Z. anal. Chem.*, 131, 17–24 (1950).
(80) Komárek, K., *Chemie (Prague)*, 4, 6–8 (1948).
(81) Komori, S., *J. Chem. Soc. Japan*, Ind. Chem. Sect., 51, 120 (1948).
(82) Korenman, I. M., Fertel'meĭster, Ya. N., and Rostokin, A. P., *Zavodskaya Lab.*, 16, 800–6 (1950).
(83) Kuffner, F., and Gruber, W., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 1177–80 (1951).
(84) Lambert, M., and Neish, A. C., *Can. J. Research*, B28, 83–9 (1950).
(85) Lang, K., and Pfleger, K., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 1174–6 (1951).
(86) Leurquin, J., and Delville, J. P., *Experientia*, 6, 274–5 (1950).
(87) *Ibid.*, pp. 6, 275–6.
(88) Levin, B., Oberholzer, V. G., and Whitehead, T. P., *Analyst*, 75, 561–3 (1950).
(89) Lévy, R., *Compt. rend.*, 230, 1958–60 (1950).
(90) Lévy, R., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 741–68 (1951).
(91) Lévy, R., and Schick, J., *Bull. soc. chim. France*, 1950, 840–2.
(92) Ley, J. de, *Mededel. Vlaam. Chem. Ver.*, 12, 27–41 (1950).
(93) Lüdy-Tenger, F., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 882–93 (1951).
(94) Mangeney, G., *Bull soc. chim. France*, 1950, 74–81.
(95) Martin, F., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 653–59 (1951).
(96) *Ibid.*, pp. 660–4.
(97) Marzadro, M., *Ibid.*, 36/37, 671–8 (1951).
(98) Mason, A. C., *Analyst*, 76, 172–6 (1951).
(99) Milazzo, G., and Paoloni, L., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 255–64 (1951).
(100) Möhlau, E., *Pharm. Zentralhalle*, 89, 334–7 (1950).
(101) Möhle, W., *Chem. Ing. Tech.*, 22, 416–17 (1950).
(102) Moelants, L., *Mededel. Vlaam. Chem. Ver.*, 12, 120–32 (1950).
(103) Müller, G., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 143–50 (1951).
(104) Nesh, F., and Peacock, W. C., ANAL. CHEM., 22, 1573 (1950).
(105) Ogg, C. L., Willits, C. O., Ricciuti, Constantine, and Connelly, J. A., *Ibid.*, 23, 911–14 (1951).
(106) Pääbo, K., and Rottenberg, M., *Acta Chem. Scand.*, 3, 1444–5 (1949).
(107) Padowetz, W., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 648–52 (1951).
(108) Parks, T. D., and Lykken, L., ANAL. CHEM., 22, 1444–6 (1950).
(109) Parks, T. D., and Lykken, L., *Petroleum Refiner*, 29, No. 8, 85–8 (1950).
(110) Pereira, A., and Serra, J. A., *Science*, 113, 387–8 (1951).
(111) Renard, M., and Deschamps, P., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 665–70 (1951).
(112) Ribas, I., and López Capont, F., *Anales real soc. españ. fís. y quím.*, 46B, 581–94 (1950).
(113) Rickard, R. R., Ball, F. L., and Harris, W. W., ANAL. CHEM., 23, 919–21 (1951).
(114) Rosenberg, S., Perrone, J. C., and Kirk, P. L., *Ibid.*, 22, 1186–7 (1950).
(115) Roth, H., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 379–92 (1951).
(116) Rüggeberg, H., *Ibid.*, 36/37, 916–23 (1951).
(117) Ruziczka, W., *Ibid.*, 36/37, 924–6 (1951).
(118) Safford, H. W., and Stragand, G. L., ANAL. CHEM., 23, 520–2 (1951).
(119) Sakaguchi, B., *Kagaku no Ryôiki (J. Japan. Chem.)*, 2, 258–62 (1948).
(120) Sakaguchi, T., *J. Pharm. Soc. Japan*, 62, 404–14 (1942).
(121) Sakamoto, S., and Hayazu, R., *Ibid.*, 70, 698–9 (1950).
(122) Sakamoto, S., Hayazu, R., and Takenaka, R., *Ibid.*, 70, 287–8 (1950).
(123) Schöniger, W., *Mikrochemie ver. Mikrochim. Acta*, 38, 132–5 (1951).
(124) Schöniger, W., *Z. anal. Chem.*, 133, 4–7 (1951).
(125) Schöniger, W., and Lieb, H., *Mikrochemie ver. Mikrochim. Acta*, 38, 165–7 (1951).
(126) Schulek, E., and Pungor, E., *Anal. Chim. Acta*, 4, 109–17 (1950).
(127) Shepard, D. L., and Jacobs, M. B., *J. Am. Pharm. Assoc.*, 40, 154–5 (1951).
(128) Siegfriedt, R. K., Wiberley, J. S., and Moore, R. W., ANAL. CHEM., 23, 1008–11 (1951).
(129) Sobotka, M., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 407–12 (1951).
(130) Stelt, C. van der, *Pharm. Weekblad*, 86, 69–74 (1951).
(131) Sterges, A. J., Hardin, L. J., and MacIntire, W. H., *J. Assoc. Offic. Agr. Chemists*, 33, 114–21 (1950).
(132) Sternglanz, P. D., Thompson, R. C., and Savell, W. L., ANAL. CHEM., 23, 1027–30 (1951).

(133) Stetten, DeWitt, Jr., *Ibid.*, 23, 1177–9 (1951).
(134) Steyermark, A., Alber, H. K., Aluise, V. A., Huffman, E. W. D., Kuck, J. A., Moran, J. J., and Willits, C. O., *Ibid.*, 23, 523–8 (1951).
(135) Stock, J. T., *Metallurgia*, 42, 48–51 (1950).
(136) Stock, J. T., and Heath, P., *Ibid.*, 42, 44 (1950).
(137) Stöhr, R., and Scheibl, F., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 362–5 (1951).
(138) Tamiya, N., *J. Japan. Biochem. Soc.*, 22, 59–62 (1950).
(139) Tanenbaum, M., and Bricker, C. E., Anal. Chem., 23, 354–7 (1951).
(140) Taussky, H. H., *J. Biol. Chem.*, 181, 195–8 (1949).
(141) Teller, J. D., *Ibid.*, 185, 701–4 (1950).
(142) Thompson, J. F., and Morrison, G. R., Anal. Chem., 23, 1153–7 (1951).
(143) Tsuzuki, Yojiro, Miwa, Makoto, and Kobayashi, Etsuro, *Ibid.*, 23, 1179–80 (1951).
(144) Unterzaucher, J., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 706–26 (1951).
(145) Viswanathan, R., *Biochem. J.*, 48, 239–40 (1951).
(146) Wagner, H., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 634–40 (1951).
(147) Wagner, H., and Bühler, F., *Ibid.*, 36/37, 641–7 (1951).
(148) Waldmann, H., *Ibid.*, 36/37, 973–88 (1951).
(149) Walter, R. N., Anal. Chem., 22, 1332–4 (1950).
(150) Wenger, P. E., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 94–103 (1951).
(151) West, P. W., Anal. Chem., 23, 176–80 (1951).
(152) White, L. M., and Long, M. C., *Ibid.*, 23, 363–5 (1951).
(153) Wiberley, J. S., *Ibid.*, 23, 656–9 (1951).
(154) Williams, H. A., *Analyst*, 75, 510–21 (1950).
(155) Williams, M. B., and Reese, H. D., Anal. Chem., 22, 1556–61 (1950).
(156) Willits, C. O., and Ogg, C. L., *J. Assoc. Offic. Agr. Chemists*, 34, 607–15 (1951).
(157) *Ibid.*, pp. 615–19.
(158) Wilson, H. N., and Straw, H. T., *J. Soc. Chem. Ind.*, 69, 79–82 (1950).
(159) Wingo, W. J., Davis, O. L., and Anderson, L., Anal. Chem., 22, 1340 (1950).
(160) Wurzschmitt, B., *Chem.-Ztg.*, 74, 419–23 (1950).
(161) Wurzschmitt, B., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 614–27 (1951).
(162) *Ibid.*, pp. 769–80.
(163) Zacherl, M. K., *Mitt. chem. Forsch.-Inst. Ind. österr.*, 4, 27–9 (1950).
(164) Zinneke, F., *Z. anal. Chem.*, 132, 175–85 (1951).